

Control of *Listeria monocytogenes* in turkey deli loaves using organic acids as formulation ingredients

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ABSTRACT The growth of *Listeria monocytogenes* in further-processed meat products has become a major concern and an important food safety issue. The meat and poultry industries have incorporated interventions such as organic acids in marinades to inhibit the growth of *L. monocytogenes*. In this study, organic acids were utilized in the raw product and as a postcook dip to determine their inhibitory effect on the growth of *L. monocytogenes* in turkey deli loaves. The turkey deli loaves were processed, cooked, cooled, inoculated with streptomycin-resistant *L. monocytogenes*, and then dipped. Treatments were potassium lactate (PL) in the raw product with sodium lactate (SL), sodium diacetate (SD) dip, PL with SL/PL/SD dip, SL with SL/SD dip, and SL with SL/PL/SD dip. There was also a

positive (inoculated) and negative (noninoculated) control, which was dipped in distilled water. Days 0, 7, 14, 21, 28, 42, and 56 were sampled for *L. monocytogenes*. There were no differences ($P > 0.05$) among the organic acid treatments in the turkey deli loaves at any time points; therefore, all of the treatments increased the lag phase of *L. monocytogenes*, extending the shelf-life of the product. However, there was a difference between the treatments and the positive control at d 7, 14, 21, 28, 42, and 56. The growth of *L. monocytogenes* increased immediately in the positive control, whereas the negative control appeared to have no growth. These organic acids can provide meat processors with a useful method for extending the lag phase of *L. monocytogenes* in ready-to-eat meat and poultry products.

Key words: *Listeria monocytogenes*, organic acid, turkey deli loaf, marinade, dip solution

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INTRODUCTION

Fresh food products of animal or plant origin can harbor *Listeria monocytogenes*. This organism has been found in raw milk, soft cheese, fresh and frozen meat, poultry, seafood products, and on fruit and vegetable products (Jay, 1996b). *Listeria monocytogenes* is sensitive to heat treatment; therefore, cooking can inactivate the pathogen (Zhu et al., 2005). Because recontamination can occur postprocessing due to handling, slicing, and packaging and because ready-to-eat (RTE) products are usually not reheated before consumption, the presence of *L. monocytogenes* poses a concern in these products (CDC, 2005; Zhu et al., 2005).

Along with heat, low water activity and an acidic environment have an adverse effect on the growth of *L. monocytogenes* (Lahti et al., 2001; Zhu et al., 2005). The organism also tolerates salt concentrations up to

10% and high nitrite concentrations (USDA, 2003; Zhu et al., 2005).

Because of the concern for human health, the US government designated *L. monocytogenes* as an adulterant (Jay, 1996b). This means that any RTE food that contains this organism can be considered adulterated and subject to recall (Jay, 1996b). The USDA Food Safety and Inspection Service has established a zero-tolerance policy for *L. monocytogenes* on RTE products because of the high fatality rate (20% in mild cases and 70% in severe cases; FSIS, 2003; FDA/CFSAN, 2006; Geornaras et al., 2006; Schultze et al., 2006).

The meat and poultry industries have incorporated intervention in their processing to meet the zero-tolerance policy. Antimicrobial additives such as organic acids are utilized in the marinades of the products to enhance flavor, increase tenderness, and prolong the shelf-life of meat products (Xargayo et al., 2001). Sodium lactate (SL) and potassium lactate (PL) and sodium diacetate (SD) are incorporated in marinades as safety hurdles to prevent the growth of some pathogenic bacteria (De Vegt, 1999). These lactates lower the water activity and pH and interfere with the metabolism

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of the bacteria (De Vegt, 1999). It has been proposed that the inhibitory mechanisms consist of an intracellular acidification (lost of homeostasis) and a specific effect of the acid (nondissociated form) on metabolic activities (Vasseur et al., 1999).

Currently, processors are utilizing organic acids in marinades to inhibit the growth of *L. monocytogenes*; however, there has been no validation on exposing *L. monocytogenes* to a hurdle combination. The objective of this research was to determine the effect of common marinade ingredients along with organic acids added to raw turkey breast meat in combination with organic acid dips on cooked slices (postprocessing) on the growth of *L. monocytogenes*. If these treatments (organic acids in the raw product and as a postcook dip) decrease or prevent *L. monocytogenes* growth, then utilization of this multihurdle approach can provide the industry with a method to maintain zero tolerance of *L. monocytogenes*.

MATERIALS AND METHODS

Organic acids were combined in the raw product and as a postcook dip for a multihurdle approach to determine the effect they have on the growth of *L. monocytogenes* on turkey deli loaves. The treatments are shown in Table 1. All treatments included a standard industry antimicrobial cocktail of sodium chloride (1.5%) and sodium tripolyphosphate (0.45%).

L. monocytogenes Growth

A streptomycin-resistant *L. monocytogenes* strain stored at -80°C in 10% glycerol was used for all experiments. Inocula were prepared by reviving from frozen storage and growing in 9.0-mL tubes of brain heart infusion (BHI) broth (Oxoid Ltd., Hampshire, UK), which were incubated at 37°C overnight. Three replicate aliquots of 1.0 mL were taken from the culture tube and placed into 3 separate 9-mL tubes of fresh BHI broth, which were also incubated overnight at 37°C . Cell numbers in suspension were enumerated by spread-plating onto duplicate BHI agar plates (Difco, Fisher Scientific Co. LLC, Pittsburgh, PA) with the addition of 1,500

mg/mL of streptomycin sulfate salt (Sigma-Aldrich Inc., St. Louis, MO) and were incubated overnight at 37°C . The cell suspension was determined to be 1.47×10^9 cfu/mL and 2.05×10^9 cfu/mL for trials 1 and 2, respectively.

Processing Turkey Deli Loaves

All processing was conducted using sterile trays, racks, and pans. To reduce variation, a total of 13.61 kg of turkey breast was supplied by Patuxent Farms (Columbia, MD) for 3 trials. Ingredients for the 2 control deli loaves and the 4 treated deli loaves were based on a 4.54-kg turkey loaf. Final concentrations of the antimicrobial ingredients were as follows: salt (1.5%; Morton Salt, Morton International Inc., Chicago, IL), sodium tripolyphosphate (0.45%; Innophos, Cranbury, NJ), PL (2%; 60% wt/wt; City Chemical LLC, West Haven, CT), and sodium lactate (2%; 60% wt/wt, Fisher Scientific Co. LLC, Pittsburgh, PA).

The pH (Accument AB15 Plus pH Meter, Fisher Scientific Inc., Rockford, IL) was measured for the antimicrobial ingredients and pretreated raw product. For each treatment formulation, 80% of the total weight of the loaf was cubed into 2.54×2.54 cm pieces and the remaining 20% was ground (MG 100-3 Waring Pro Meat Grinder, Waring Consumer Products, East Windsor, NJ) with a 7-mm blade. The ground and cubed meat was marinated with the above raw ingredients separately by treatment in a vacuum tumbler (LT5 Koch Tumbler, Lance Industries, Allenton, WI) 4.54 to 13.61 kg, 40 rpm, 23 mm/Hg, 2 h, 4°C . After tumbling, the loaves were stored at 4°C for 3 h in the cooler to allow the meat to equilibrate. The pH of the postmarinated (antimicrobial ingredients) raw product was then determined. The loaves were then manually stuffed (F. Dick Tabletop Piston Stuffer, 13.61-kg capacity, Fredrick Dick Corp., Farmingdale, NY) by treatment into 10.16 cm-diameter fibrous casings (EZ Peel Fibrous Casing, Viskase Companies Inc., Willowbrook, IL) and cooked (Table 2) in a smokehouse (Alkar Smokehouse, DEC International, Lodi, WI) to an internal temperature of 71°C measured by Multitrip (multiuse temperature recorder, Temprecord, Modesto, CA). After cooking,

Table 1. Formulation for the marinade ingredients and postcook dip for turkey deli loaves¹

Treatment	Antimicrobial in formulation	Postcook dip
1	PL ² (2%)	SL (3.6%), SD ³ (0.25%)
2	PL (2%)	SL (3.6%), PL (3.6%), SD (0.25%)
3	SL ⁴ (2%)	SL (3.6%), SD (0.25%)
4	SL (2%)	SL (3.6%), PL (3.6%), SD (0.25%)
Control ⁵	Salt ⁶ (1.5%), STPP ⁷ (0.45%)	Distilled water

¹Salt (1.5%) and sodium tripolyphosphate (STPP; 0.45%) were added in all formulations.

²Potassium lactate (PL), 60% wt/wt, City Chemical LLC, West Haven, CT.

³Sodium diacetate (SD), Spectrum Chemical Mfg. Corp., Gardena, CA.

⁴Sodium lactate (SL), 60% wt/wt, Fisher Scientific Co. LLC, Pittsburgh, PA.

⁵Positive (inoculated) and negative (noninoculated) control.

⁶Salt, Morton Salt, Morton International Inc., Chicago, IL.

⁷Sodium tripolyphosphate, Innophos, Cranbury, NJ.

Table 2. Dry bulb temperatures, wet bulb temperatures, time, and smoke setting for each step used in the turkey deli loaf smoke cycle

Step	Dry bulb temperature (°C)	Wet bulb temperature (°C)	Time (min)	Smoke
1	53.9	-17.8	30	Off
2	65.6	46.1	120	Off
3	76.7	58.9	60	Off
4	85	68.9	Until 71.1°C internal temperature	Off
5	-17.8	-17.8	15 (cold shower)	Off

the loaves were then cooled to an internal temperature of 4°C, the casings were removed, and the deli loaves were sliced (9512 12" Max Manual Meat Slicer, Univex Corp., Salem NH) into 2-mm portions.

Microbiological Analysis

Each slice was inoculated by dripping 10 µL of a 100 cell/mL cell suspension (10^5 cells of *L. monocytogenes* per link) onto the surface and spreading with a sterile plastic inoculating loop. Slices were allowed to air dry (5 min) on autoclaved foil before dipping them into the postcook marinade dip.

Eight beakers, each with 500 mL, were prepared for each postcook treatment dip. The solution pH and water activity (TH-500 A_w Sprint, Novasina, Pfäffikon, Switzerland) was noted for each treatment. After immersion for 60 s, 5 links were left on sterile foil and allowed to air dry at room temperature for approximately 15 to 20 min to allow for bacterial attachment. Three slices for every treatment were placed together in a Whirl-Pak bag (21 links per treatment, 7 bags per treatment, totaling 126 links and 42 bags; Whirl-Pak Bags, Nasco, Fort Atkinson, WI) and placed at 4°C for 0, 7, 14, 21, 28, 42, or 56 d before analysis.

Noninoculated negative control slices were dipped in sterile water for 60 s, allowed to dry 15 to 20 min, packaged in Whirl-Pak bags, and immediately placed at 4°C. Positive control slices were inoculated with *L. monocytogenes*, allowed to dry 5 min, dipped in sterile water for 60 s, allowed to dry for approximately 15 to 20 min, packaged, and stored at 4°C. The treated slices and positive and negative control slices were tested for *L. monocytogenes* on d to ensure proper inoculation was attained.

For each testing time point, 1 slice was removed from each bag, placed in a filter stomacher bag (Filtered Homogenizer Bags, 3M, St. Paul, MN) with 50 mL of sterile PBS (Pierce, Thermo Fisher Scientific Inc., Rockford, IL) and homogenized (400 Circulator Stomacher, Seward, West Sussex, UK) for 2 min at 230 rpm. The samples were then further diluted, 1 mL into 9 mL of sterile PBS. Because the samples contained fat and meat particles, they were filtered through a syringe (10-mL syringe, VWR International Inc., Bristol, CT with a 1.2-µm filter, Minisart single-use syringe filter, sterile-ethylene oxide, nonpyrogenic, Sartorius, Edgewood, NY) and dispensed into a sample cup before spiral plating. Dilutions 1 and 4 were plated onto

BHI agar with 1,500 mg/mL of streptomycin using a spiral plater (AP4000 Spiral Biotech, Automated Spiral Plater, Advanced Instrument Inc., Norwood, MA) with a vacuum (VS2 Spiral Biotech, Vacuum Source, Advanced Instrument Inc.) according to the instructions of the manufacturer. Plates were incubated at 35°C for 48 h. Resulting colonies were counted using an automated colony counter (530 Spiral Biotech Automatic Plate Counter, Advanced Instruments Inc.).

Statistical Analysis

The experiment was repeated twice (2 trials) with 3 replications within each trial. No replication × treatment or trial × treatment interactions were noted; therefore, the data were pooled by treatment and day. Data were analyzed by day and over time using the PROC GLM procedure of SAS (SAS Institute, 1993). Means were separated by Duncan's multiple range test. Significant differences among treatment groups were determined at a level of $P < 0.05$.

RESULTS AND DISCUSSION

Table 3 shows the solution pH and water activity data for the antimicrobial formulations and postcook dips. The pH for the antimicrobial formulations for the PL, SL, and control were 7.89, 7.56, and 7.98, respectively, and the pH for the SL/SD, SL/PL/SD, and distilled water dips were 5.32, 5.63, and 7.00, respectively. Organic acids when added to a product lower the pH but can also lower the water activity (DeVegt, 1999). Previous studies concluded that organic acids inhibit the growth of *L. monocytogenes* by lowering the water activity (Debevere, 1989). The water activity for the dips containing organic acids (SL/SD and SL/PL/SD) was lower than the sterile distilled water (0.85, 0.80, and 1.00, respectively). However, the water activity was not measured on the deli slices in this study.

The growth of *L. monocytogenes* on slices treated with the organic acid combinations is shown in Table 4. For each time point (d 0 to 56), the PL with SL and SD dip (PL-SL/SD); PL with SL, PL, and SD dip (PL-SL/PL/SD); SL with SL and SD dip (SL-SL/SD); and SL with SL, PL, and SD dip (SL-SL/PL/SD) treatments were not significantly different. *Listeria monocytogenes* growth for the control increased ($P < 0.05$) compared with the treated samples on d 7 and 14. By d 21, there was a 4-log increase between the control and the treated

Table 3. Marinade solution pH, pre- and postmarination pH values of the raw turkey deli loaves, and dip solution pH and water activity (A_w) for the control and treatments

Treatment	Solution pH	Premarination pH	Postmarination pH	Solution A_w
Ingredient				
PL ¹	7.89	6.34	6.13	
SL ²	7.56	6.15	6.15	
Control ³	7.98	6.08	6.08	
Dip				
SL/SD ⁴	5.32			0.85
SL/PL/SD ⁵	5.63			0.80
Control ⁶	7.00			1.00

¹Potassium lactate (2%), salt (1.5%), sodium tripolyphosphate (STPP; 0.45%), and water.

²Sodium lactate (2%), salt (1.5%), STPP (0.45%), and water.

³Salt (1.5%), STPP (0.45%), and water.

⁴Sodium lactate (3.6%), sodium diacetate (0.25%), and water.

⁵Sodium lactate (3.6%), potassium lactate (3.6%), sodium diacetate (0.25%), and water.

⁶Distilled water.

slices and that difference was maintained for the subsequent time points. This confirms that the addition of organic acids (weak acids) in foods is a beneficial food preservation technique.

When lactates are used in meat products, the pH is lowered, creating an environment that has an adverse effect on the growth of *L. monocytogenes* (Doyle, 1999). The adverse pH affects 2 aspects of a respiring microbial cell: the functioning of its enzymes and the transport of nutrients into the cell (Jay, 1996a). Organic acids are more efficient in their nondissociated form and can be correlated with their dissociation constant. The dissociation constant values for SL, PL, and SD are 3.86, 3.86, and 3.58, respectively (Benjamin, 1998; Vasseur et al., 1999; Berg et al., 2002). Weak acids in their undissociated form have a greater permeability of the cell (Ita and Hutkins, 1991). This could explain the current data, in which the lactic acid that was added in formulation and as a dip to the turkey deli loaves inhibited the growth of *L. monocytogenes* throughout the 56 d.

Sodium lactate (3.5%) and sodium acetate (0.5%) were added individually to cooked ham (Blom et al., 1997). After microbial analysis, both organic acids at these concentrations inhibited growth of the pathogen

(Blom et al., 1997). Blom et al. (1997) also applied a hurdle technique to the ham. They combined 1.75% SL and 0.25% sodium acetate in the presence of 2.75% salt, which gave them complete growth inhibition in pH ranging from 5.5 to 6.3 (Blom et al., 1997). Throughout the entire 5 wk of storage at 4°C, complete inhibition of *L. monocytogenes* was observed in the cooked, sliced ham formulated with the acid mixture, whereas there was good growth of the pathogen in the control samples (Blom et al., 1997). The current study had similar results to the work of Blom et al. (1997) because when combining multiple organic acids together at different stages of production with a salt content of 1.5%, the growth was prevented.

Table 4 represents *L. monocytogenes* growth over time on slices treated with organic acids and on the control. The growth of *L. monocytogenes* for each treatment containing organic acids (PL-SL/SD, PL-SL/PL/SD, SL-SL/SD, and SL-SL/PL/SD) applied to the turkey deli loaves was similar over the 56 d. On the other hand, *L. monocytogenes* growth for the control increased between each 7-d time point until it reached its stationary phase at d 21. From d 21 to d 56, the colony count was similar. Buchanan et al. (1993) con-

Table 4. Streptomycin-resistant *Listeria monocytogenes* (cfu/mL) and SEM within a day sampled on d 0 to 56 on turkey deli loaves (n = 210) stored at 4°C when organic acids were applied in the raw product combined with a postcook dip¹

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56
1 ²	4.16 ^a ± 0.14	4.22 ^b ± 0.13	4.25 ^b ± 0.18	4.31 ^b ± 0.10	4.34 ^b ± 0.14	4.26 ^b ± 0.18	4.43 ^b ± 0.08
2 ³	4.32 ^a ± 0.15	4.31 ^b ± 0.14	4.39 ^b ± 0.15	4.24 ^b ± 0.11	4.32 ^b ± 0.11	4.31 ^b ± 0.12	4.27 ^b ± 0.14
3 ⁴	4.47 ^a ± 0.12	4.63 ^b ± 0.11	4.54 ^b ± 0.12	4.46 ^b ± 0.11	4.51 ^b ± 0.10	4.36 ^b ± 0.09	4.22 ^b ± 0.13
4 ⁵	4.24 ^a ± 0.15	4.33 ^b ± 0.14	4.24 ^b ± 0.09	4.34 ^b ± 0.09	4.39 ^b ± 0.15	4.16 ^b ± 0.16	4.06 ^b ± 0.15
Control+ ⁶	3.29 ^{b,z} ± 0.16	5.3 ^{a,y} ± 0.14	7.95 ^x ± 0.11	8.68 ^{a,w} ± 0.11	8.59 ^{a,w} ± 0.15	8.69 ^{a,w} ± 0.04	8.45 ^{a,w,x} ± 0.14

^{a,b}Least squares means within a column with different superscripts differ ($P < 0.05$).

^{w,z}Least squares means within a row with different superscripts differ ($P < 0.05$).

¹Salt (1.5%) and sodium tripolyphosphate (STPP; 0.45%) were added in all formulations.

²Treatment 1: potassium lactate (2%)-sodium lactate (3.6%) and sodium diacetate (0.25%).

³Treatment 2: potassium lactate (2%)-sodium lactate (3.6%), potassium lactate (3.6%), and sodium diacetate (0.25%).

⁴Treatment 3: sodium lactate (2%)-sodium lactate (3.6%) and sodium diacetate (0.25%).

⁵Treatment 4: sodium lactate (2%)-sodium lactate (3.6%), potassium lactate (3.6%), and sodium diacetate (0.25%).

⁶Control+: inoculated control loaf dipped in distilled water.

cluded after observing the effect of acidulants on *L. monocytogenes* growth that when using organic acids, the inhibition of *L. monocytogenes* is enhanced (Buchanan et al., 1993).

The treatments that contained the organic acids extended the lag phase increasing the shelf-life of the turkey deli loaves. The treatments were not significantly different; therefore, utilizing these lactate combinations in the industry can help prevent the growth of *L. monocytogenes*. Further studies should be completed comparing the inhibitory effect of a SL/SD treatment, SL/SA treatment, and SL/SD/SA treatment.

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